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BRIEFER ARTICLES

EFFECT OF ZINC AND IRON COMPARED WITH THAT OF URANIUM AND COBALT ON GROWTH OF ASPERGILLUS

It has been shown in a preceding publication[1] that treatment of Pfeffer's nutrient solution with calcium carbonate at an elevated temperature removes from the solution the last traces of iron, zinc, and probably the other heavy metals to a high degree. It has also been shown that Pfeffer's solution so treated supports but a minimal growth of *Aspergillus niger*, but that the addition of both iron and zinc results in a phenomenal increase in growth as measured by the dry weight formed. The evidence presented, while showing that the presence of zinc in the cultural solution is just as necessary for *A. niger* as the presence of iron, did not include the action of other heavy metal salts.

Theoretically the extension of these observations to include other heavy metal salts is of importance, inasmuch as some light would be thrown on the parts played by iron and zinc salts in the metabolism of this fungus. If other heavy metal salts can replace either or both iron and zinc salts, as is commonly stated, then iron and zinc are not essential for the growth of *A. niger* and play the part of "chemical stimulants." If they cannot be replaced by other heavy metal salts, then they possess properties that remove them from this category and place them among the elements essential for the growth of *A. niger.*

For the purpose of this comparison only $Co(NO_3)_2+6H_2O$ and $UO_2(NO_3)UO_2(NO_3)_2+6H_2O$, both Kahlbaum reagents, were available, and these were therefore used. The method used has already been described (see footnote 1).

The results with $Fe_2(PO_4)_2+4H_2O$ and with $ZnSO_4+7H_2O$ apparently point to an incomplete action of the treatment with calcium carbonate, especially in reference to the removal of iron, since the cultures to which only zinc was added gave dry weights much higher than usual (see footnote 1). In the series containing both iron and zinc the usual phenomenal increases in dry weight occurred. Also regarding acidity it will be noticed, what has already been pointed out, that a parallelism seems to

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[1] Steinberg, R. A., A study of some factors in the chemical stimulation of the growth of *Aspergillus niger*. Amer. Jour. Bot. **6**:330. 1919.

TABLE I

EFFECT ON GROWTH OF *A. niger* OF ADDITION OF IRON, ZINC, COBALT, AND URANIUM SALT TO TREATED PFEFFER SOLUTION (STRAIN W, 12)*

SOLUTION	YIELD	PH	SPORULATION
	$FePO_4$		
$FePO_4$/flask	(gm.)		
0 mg.	0.049	3–4	Fair
1	0.044	3–4	Fair
5	0.046	3–4	Fair
20	0.047	3–4	Fair
50	0.072	3–4	Fair
	$ZnSO_4+7H_2O$		
Zn/liter			
0 mg.	0.026	3–4	Fair
1	0.060	3–4	Fair
5	0.061	3–4	Fair
20	(0.267)	2–3	Good
50	(0.362)	2–3	Practically sterile
	1.0 mg. $FePO_4$/flask+zinc†		
Zn/liter			
0.1 mg.	0.377	2–3	Good
0.5	0.771	1–2	Fair
1	0.694	1–2	Fair
10	0.688	1–2	Fair
20	0.723	1–2	Fair
50	0.759	1–2	Fair
	1.0 mg. $FePO_4$/flask+$Co(NO_3)_2$		
$Co(NO_3)_2$/flask			
0.1 mg.	0.072	3–4	Practically sterile
0.5	0.058	3–4	Practically sterile
1	0.053	3–4	Practically sterile
10	0.049	3–4	Practically sterile
20	0.033	3–4	Practically sterile
50	0.026	3–4	Practically sterile
	1.0 mg. $FePO_4$/flask+$UO_2(NO_3)_2$		
$UO_2(NO_3)_2$/flask			
0.1 mg.	0.048	3–4	Practically sterile
1	0.062	3–4	Practically sterile
5	0.062	3–4	Practically sterile
10	0.058	3–4	Practically sterile
20	0.069	3–4	Practically sterile
50	0.080	3–4	Practically sterile

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* The compounds used in the studies were Baker's "analyzed" KH_2PO_4; $ZnSO_4+7H_2O$; and $CaCO_3$; and Merck's "reageant" NH_4NO_3; $MgSO_4+7H_2O$ and "highest purity" sucrose. All other compounds were the Kahlbaum.

† The zinc ($ZnSO_4+7H_2O$) was added to these cultures 3.5 days after inoculation.

TABLE I—*Continued*

Solution	Yield	Ph	Sporulation
	0.1 mg. Zn/liter+Co $(NO_3)_2$		
$Co(NO_3)_2$/flask	(gm.)		
0.1 mg	0.075	3–4	Practically sterile
0.5	0.103	3–4	Fair
1	0.093	3–4	Fair
10	0.190	3–4	Excellent
20	0.180	3–4	Excellent
50	0.192	3–4	Excellent
	0.1 mg. Zn/liter+$UO_2(NO_3)_2$		
$UO_2(NO_3)_2$/flask			
0.1 mg	0.062	3–4	Practically sterile
1	0.053	3–4	Practically sterile
5	0.053	3–4	Practically sterile
10	0.138	3–4	Fair
20	(0.462)	2–3	Fair
50	0.095	3–4	Practically sterile

exist between increase in dry weight, increased acidity, and decrease in sporulation.

Examination of the data obtained with $Co(NO_3)_2+6H_2O$ and with $UO_2(NO_3)_2+6H_2O$ shows the surprising result that the phenomenal increases in dry weight ascribed to these metals do not occur.[2] The increases in dry weight, excepting for a few cases (zinc plus cobalt salts), are within the experimental error of the observations. The unincreased acidity of the cultures supports the viewpoint that no increases in growth had been obtained. As concerns sporulation, there seems to be no regularity in the cultures containing cobalt or uranium, and while it is interesting to note that in the last series, containing only cobalt and uranium, sporulation took place, it would be an error to suppose that this necessarily implies that neither zinc nor iron is needed by *A. niger* for spore formation. The probable explanation is the presence of traces of iron and of zinc in the cobalt and uranium salts.

The lack of any marked increases in dry weight brought about by the addition of cobalt and uranium salts to the cultural solution is entirely contrary to the results already recorded in the literature. The explanation apparently best fitting the facts is that partial replacement may occur and does occur only when iron and zinc are not present in too low amounts. It would seem certain, considering the many described cases of increased growth caused by heavy metal salts, that the contamination of the "stimulants" with iron and zinc could not have occurred without some exceptions if contamination of the "stimulants" were the cause.

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[2] LE PIERRE, C., Remplacement du zinc par l'uranium dans la culture de l'*Aspergillus niger*. Compt. Rend. **156**:156. 1179. 1913; RICHARDS, H. M., Die Beeinflussung des Wachstums einiger Pilze durch chemische Reize. Jahrb. Wiss. Bot. **30**:665. 1897.

It is not impossible that the action of iron and of zinc and the action of the other so-called "chemical stimulants" will be found to be intimately connected with the increased acidity of the nutrient solution. This hypothesis has already been discussed in a former paper. The assumption of a direct action of the "chemical stimulants" must be dropped, however, since these experiments make apparent that their effects are in some manner related to those of iron and zinc, which latter are directly correlated with acid production by the organism. This explanation can also be extended to include the alkaloids, since the extremely high concentrations in which they are effective in bringing about increased growth would eliminate from consideration their action as alkaloids, and their chemical properties as free base and as acid salts are well known. The action of the chemically inert "stimulants," such as ether, chloroform, etc., may in the same manner be laid to the disturbance of the respiration processes leading to an accumulation of organic acids. The fact that free oxalic acid, in common with other acids, can bring about this increased growth, etc., renders this supposition not improbable, in spite of the fact that it is commonly stated that the products of catabolism of an organism exercise a harmful effect on its growth.[3] Clearly we have here a case in which a product of the metabolism causes an acceleration of the rate of growth of the organism.

These studies must be looked upon, therefore, as a confirmation of the conclusions arrived at by RAULIN (Études chimiques sur la végétation. Ann. Sci. Nat. Bot. V. **11**: 93. 1869) and by JAVILLIER (La Présence et la rôle du zinc chez la plantes. Theses. Paris. 1908) that both iron and zinc are essential for the growth of *Aspergillus niger*, and the assumption must be made that when iron and zinc are present in favorable amounts a partial replacement of either or both these elements can occur by the so-called "chemical stimulants."—R. A. STEINBERG, *Bureau of Plant Industry, Washington, D.C.*

[3] PFEFFER, W., Physiology of Plants. I. Oxford. 1897.